

DURATION OF HERBIVORE REMOVAL AND ENVIRONMENTAL STRESS AFFECT THE ECTOMYCORRHIZAE OF PINYON PINES¹

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Abstract. The mutualistic mycorrhizal symbionts of plants have shown mixed responses to herbivory: they either decrease, increase, or show no measurable change. We examined the ectomycorrhizal responses of pinyon pine (*Pinus edulis*) exposed to 1 yr of simulated herbivory in two environments, one stressful and one less so. We also compared levels of ectomycorrhizal colonization and conelet production in pinyons from which an important insect herbivore had been removed for either 1 or 10 yr. Pinyons that grew in more stressful cinder soils experienced significant reductions (19%) in ectomycorrhizal colonization following 1 yr of simulated herbivory while pinyons growing in less stressful sandy loam soils did not. These results indicate that the degree of environmental stress experienced by a plant could affect whether mycorrhizal reductions result from herbivory. In addition the reductions in ectomycorrhizal colonization that resulted from chronic herbivory remained for a full year following herbivore removal even though conelet production increased 250-fold in the same time period. Our findings regarding the role of environmental stress and duration of herbivory in affecting mycorrhizal responses may help explain the variable responses found in other systems.

Key words: *ectomycorrhizae; environmental stress; herbivory; herbivore removal; Pinus edulis.*

INTRODUCTION

Although aboveground herbivores can have positive effects on plants (Whitham et al. 1991), their consumption of plant tissues often negatively affects plant performance (Louda et al. 1990). One indirect impact herbivory can have on plants is to affect the symbiotic relationship between a plant and its mycorrhizal fungi (e.g., Wallace 1981, Bethlenfalvai and Dakessian 1984, Trent et al. 1988, Gehring and Whitham 1991). Mycorrhizal fungi can enhance host-plant nutrient uptake and pathogen resistance (Harley and Smith 1983), leading to increased plant growth and reproduction in the greenhouse (Harley and Smith 1983) and in the field (Stanley et al. 1993). The fungi also benefit from this mutualism, receiving photosynthate from host plants (Harley and Smith 1983).

Because both aboveground herbivores and mycorrhizal fungi require energy from plants, they are likely to interact with one another. The studies that have examined these interactions have produced various results: mycorrhizal colonization declined following herbivory in 23 plant species, showed no relationship with herbivory in 10 plant species, and was positively associated with herbivory in two plant species (reviewed in Gehring and Whitham 1994a). Several factors could contribute to these differences in response, including: (1) differences in the intensity or duration of herbivory, (2) variation between fungal and/or plant species in their tolerance of herbivory, and (3) variation in en-

vironmental parameters such as soil moisture and nutrient content that are likely to influence the above factors.

Previous studies have shown that susceptible pinyon pines (*Pinus edulis* Engelm.) suffering chronic herbivory by two different insect species, a stem- and cone-boring moth (*Dioryctria albovittella*) and a needle-feeding scale (*Matsucoccus acalyptus*), had $\approx 30\%$ lower levels of ectomycorrhizal colonization than pinyons naturally resistant to these insects (Gehring and Whitham 1991, Del Vecchio et al. 1993). When either of the insects were experimentally removed for several years, levels of ectomycorrhizal colonization rebounded to levels similar to those of resistant trees, demonstrating that the herbivores were responsible for the mycorrhizal reductions. These ectomycorrhizal reductions are potentially important; greenhouse experiments showed that a 30% drop in ectomycorrhizae led to a 20% decrease in seedling shoot mass (Gehring and Whitham 1994b).

In the present study we address two questions regarding the ectomycorrhizae-plant-herbivore interaction in pinyon pines: (1) Is chronic herbivory necessary to negatively affect ectomycorrhizae, or will a single season of simulated herbivory produce the same effect? and (2) Does the negative effect of herbivory on mycorrhizae disappear as soon as the herbivore is removed or does the effect persist? We addressed the first question in two pinyon habitats: an area composed of cinder soils low in moisture and nutrients, and a nearby sandy loam site higher in nutrients and moisture (Mopper et al. 1991, Gehring and Whitham 1994b) (Table 1). Pin-

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TABLE 1. Comparisons of pinyon pine sites in Arizona, USA. All comparisons are significant at $P < 0.05$.

	Cinder	Sandy loam
Phosphate ($\mu\text{g/g}$)*	4.45	12.20
Soil moisture (% water)*	5.61	9.39
NO_3 mineralization ($\mu\text{g}\cdot\text{g}^{-1}\text{ soil}\cdot\text{d}^{-1}$)*	0.015	0.147
NH_4 mineralization ($\mu\text{g}\cdot\text{g}^{-1}\text{ soil}\cdot\text{d}^{-1}$)*	-0.021	0.062
Moth herbivory (% shoot mortality)*	7.03	0.04
Growth (1990 shoot length; mm)* \ddagger	23.20	28.90
Conelet production (mean no. cones per tree per site) \ddagger	185.00	300.00
Water stress (xylem pressure; MPa) \S	-2.830	-2.380

* Data represent the mean values for four cinder and four sandy loam sites including those used in this study (adapted from Gehring and Whitham 1994b).

\ddagger Shoot length measurements are the average of four terminal and four lateral shoots from each of eight trees at each of four cinder and four sandy loam sites.

\ddagger Mean of total conelet production per tree in 1988 based on a minimum of 9 trees/site (extrapolated from Christensen and Whitham 1991: Fig. 1).

\S Mean water stress for pinyons growing in one cinder and one sandy loam site (extrapolated from Mopper et al. 1991: Fig. 2).

yons growing in cinder soils had higher levels of insect herbivory (Mopper et al. 1991, Gehring and Whitham 1994b), higher levels of water stress (Mopper et al. 1991), higher levels of ectomycorrhizal colonization (Gehring and Whitham 1994b), lower levels of defensive resins (N. S. Cobb, *personal communication*), lower growth rates (Gehring and Whitham 1994b), and lower cone production (Christensen and Whitham 1991) than pinyons growing in sandy loam (Table 1). Addressing question 1 in both a cinder and a sandy loam site allowed us to examine whether the ectomycorrhizal responses to short-term herbivory differed between pinyons growing in a stressful and a more benign environment.

METHODS

Experiment 1

To examine whether chronic herbivory was necessary to cause ectomycorrhizal declines in pinyons at a cinder site, we selected 20 moth-resistant trees of similar size in 1992 and exposed 10 of them to one season of clipping designed to mimic stem- and cone-boring moth herbivory. The remaining 10 trees served as unclipped controls. Moth-resistant trees that experienced chronically low levels of *Dioroctria* herbivory (<5% annual shoot mortality) and differed genetically from moth-susceptible trees (Mopper et al. 1991) were used in this experiment for two reasons. First, the use of resistant trees in simulated herbivory experiments allowed us to better control the overall level of tissue loss for each tree. Second, the use of resistant trees reduced the potential impacts of prior herbivory. All trees were growing in cinder soils near Sunset Crater National Monument (35°22' N, 111°32' W), northeast of Flagstaff, Arizona, USA.

To determine if the trees differed significantly in ectomycorrhizal colonization prior to clipping, we collected root samples and determined the percentage of ectomycorrhizal colonization for each of the trees in April. Approximately 40 cm of root containing >100 short roots was collected from the northeast side of each tree by digging with a trowel to a maximum depth of 20 cm. The percentage of short root tips that were actively ectomycorrhizal was determined using the characteristics described by Harvey et al. (1976).

Trees were clipped to mimic moth herbivory the following June. The stem- and cone-boring moth feeds on pinyon shoots as they elongate in the spring, nearly always killing the shoot. Moth herbivory can destroy up to 50% of a plant's new shoots, and the moth's preference for terminal shoots results in a bushy architecture and virtual elimination of female cone production (Whitham and Mopper 1985). We clipped 25% of each experimental tree's newly growing shoots at their bases using hand clippers. This level of simulated herbivory was chosen because it reflected the observed mean of 25% shoot mortality caused by moths on heavily infested susceptible trees at the cinder site during the past 10 yr. We mimicked the moth's preference for terminal shoots by clipping a greater percentage of terminal than lateral shoots (60% vs. 40%, respectively). We also determined levels of natural moth herbivory for each tree by estimating the percentage of shoots destroyed by the moth. Natural levels of moth herbivory on the trees used in this study were low because we chose resistant trees that had low levels of herbivory.

In August (the next pulse of root and mycorrhizal growth), we again collected root samples and determined the percentage of ectomycorrhizal colonization. Because previous studies have shown that levels of ectomycorrhizal colonization in pinyons are similar in April and August (Gehring and Whitham 1991, Del Vecchio et al. 1993), we did not expect the time of year to have an effect in this study. Experimental and control tree herbivory and ectomycorrhizal data were compared using Student's *t* tests (Zar 1984). The herbivory data were natural log-transformed prior to analysis due to variance inequalities.

We performed the same experiment on pinyons growing in sandy loam soils near Walnut Canyon National Monument, east of Flagstaff, Arizona, USA, in 1993. Twenty-eight similarly-sized trees were selected; 14 trees were clipped as described above, and 14 trees served as unclipped controls. Levels of moth herbivory are extremely low on pinyons growing in sandy loam (Mopper et al. 1991, Gehring and Whitham 1994b; Table 1) and were not measured in this study.

Experiment 2

To determine how quickly pinyon ectomycorrhizae recovered following chronic moth herbivory in cinder soils, we removed moths from seven moth-susceptible

pinyons in August of 1992. In the year prior to moth removal, the number of shoots destroyed by this insect were ≈ 10 times higher in these chronically attacked trees than in the average uninfested tree. Moths were removed using a single application of the systemic insecticide Cygon following the protocol of Whitham and Mopper (1985). In August 1993, when the trees had been virtually moth free for 1 yr, we collected root samples and determined levels of ectomycorrhizal colonization as described above. We also measured the ectomycorrhizal colonization of seven moth-susceptible trees that continued to experience moth herbivory in 1993, and of eight moth-susceptible trees from which moths had been removed annually since 1983. By comparing levels of ectomycorrhizal colonization in these three groups (1 yr moth-free, 10 yr moth-free, and controls), we sought to determine how rapidly ectomycorrhizae could rebound after herbivore removal. We assessed the effectiveness of the insecticide by measuring levels of moth herbivory for all trees as described for Experiment 1. Because moths cause dramatic reductions in female cone production (Whitham and Mopper 1985), we counted the number of female conelets produced by each tree as a second indicator of the effectiveness of moth removal.

Levels of moth herbivory and ectomycorrhizal colonization were compared for the three groups using a one-way analysis of variance followed by a Tukey's test to determine the location of differences (Zar 1984). Because of variance inequalities that could not be adjusted using transformations, conelet production was compared among groups using a Kruskal-Wallis test followed by a Dunn's multiple-comparison test (Zar 1984).

RESULTS

Experiment 1

For trees growing in cinder soils, a single pulse of simulated herbivory at a level comparable to natural herbivory caused a significant 19% reduction in ectomycorrhizal colonization. Although there were no differences between the two groups of trees in ectomycorrhizal colonization prior to clipping ($t = -0.730$, $P = 0.475$; Fig. 1A), pinyons exposed to one season of clipping had significantly lower levels of ectomycorrhizal colonization than unclipped controls ($t = -2.263$, $P = 0.036$; Fig. 1A). Levels of ectomycorrhizal colonization in unclipped trees were comparable to those observed in previous years (Fig. 1A: dashed line). Natural levels of moth herbivory in the two groups were similar ($3.36 \pm 0.50\%$ shoot mortality for clipped trees, and $4.93 \pm 1.02\%$ for control trees, mean ± 1 SE; $t = 1.137$, $P = 0.271$) indicating that the difference between groups was due to clipping rather than natural herbivory.

Although a single pulse of simulated herbivory negatively affected the ectomycorrhizae of pinyons grow-

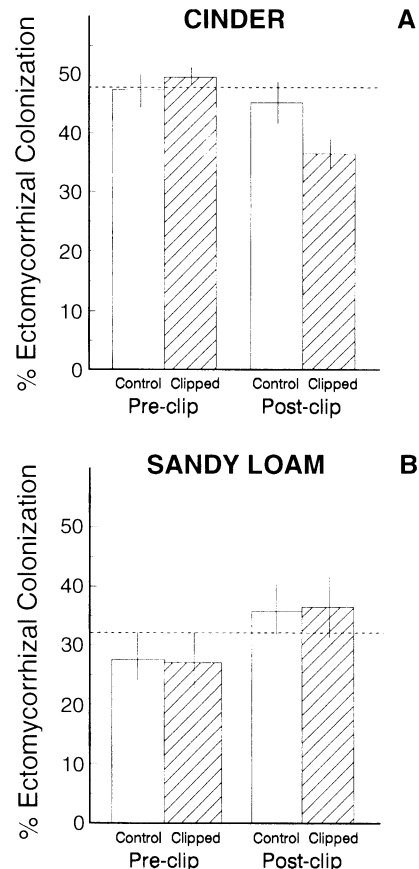


FIG. 1. Percentage of ectomycorrhizal colonization of pinyons growing in cinder (A) or sandy loam (B) soils before and after half of them were clipped to mimic moth herbivory. Data are reported as means ± 1 SE. The dashed lines represent the mean levels of ectomycorrhizal colonization of similarly sized trees over a 2–3 yr period.

ing in cinders, identical experiments conducted on pinyons growing in sandy loam resulted in no significant impact on ectomycorrhizae (Fig. 1B). Experimental and control-group pinyons at the sandy loam site did not differ from one another in levels of ectomycorrhizal colonization before clipping ($t = -0.082$, $P = 0.935$) or after clipping ($t = -0.136$, $P = 0.893$). Again, levels of ectomycorrhizal colonization were similar to those previously documented at this site (Fig. 1B: dashed line).

Experiment 2

For trees growing in cinder soils, 10 yr of moth removal resulted in significant rebounds in ectomycorrhizal colonization, while 1 yr of moth removal did not. Measurements of the percentage of shoot mortality due to moth herbivory demonstrated that our moth removal was effective (Fig. 2A). Both groups of insecticide-treated trees (1 yr and 10 yr moth free) had significantly lower levels of shoot mortality than control trees ($F_{2,19} = 51.116$, $P = 0.0001$; Fig. 2A). Pinyons

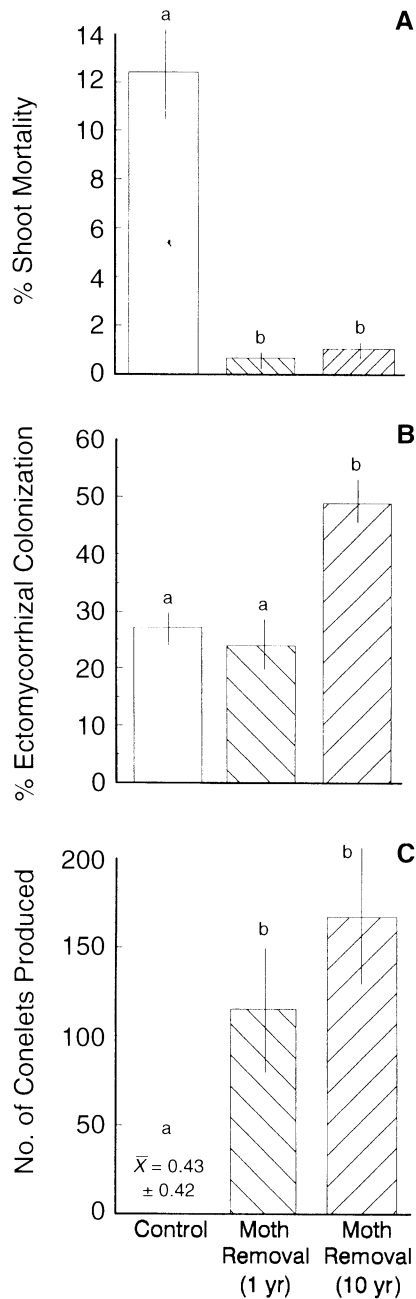


FIG. 2. Moth herbivory (A), percentage of ectomycorrhizal colonization (B), and conelet production (C) of susceptible control trees and susceptible trees from which moths had been removed for 1 or 10 yr. Data are reported as means \pm 1 SE. Different lower case letters above the bars indicate that treatments differ significantly at $P < 0.05$ (ANOVA).

from which moths had been removed for 1 yr had levels of ectomycorrhizal colonization similar to control trees that continued to experience moth herbivory (Fig. 2B). Both of these groups had significantly lower levels of ectomycorrhizal colonization than trees from which moths had been removed for 10 yr ($F_{2,19} = 16.664$, $P = 0.0001$; Fig. 2B). These results indicate that, for

pinyons in cinder soils, moth herbivory caused reductions in ectomycorrhizal colonization that remained for some time following herbivore removal.

While 1 yr of moth removal did not affect ectomycorrhizae, it did have a significant effect on female conelet initiation. Trees that were free of moths for 1 or 10 yr produced substantially more conelets than control trees, (Kruskal-Wallis test statistic = 12.504, $P = 0.002$; Fig. 2C). These results suggest that energy allocation to conelet production has a higher priority than energy allocation to ectomycorrhizae during the period immediately following herbivore removal.

DISCUSSION

Negative effects of simulated herbivory on ectomycorrhizae in cinder but not sandy loam soils

Trees exposed to one season of simulated herbivory in cinder soils experienced significant reductions in ectomycorrhizal colonization, while trees exposed to the same herbivory in sandy loam soils did not. Several hypotheses could explain this pattern. First, this result could be due to differences in the years in which the two sites were sampled. However, we feel that this is unlikely because the ectomycorrhizal levels observed in this study were comparable to those previously documented for similarly sized trees at these sites. For example, 1990 and 1991 ectomycorrhizal levels on resistant trees at the cinder site ranged from 45 to 52% (Gehring and Whitham 1991, 1994b) while levels in this study ranged from 45 to 50%. Similarly, 1991 ectomycorrhizal levels of trees at the sandy loam site averaged 32% (Gehring and Whitham 1994b), while those in this study also averaged 32%.

Second, pinyons growing at the two sites differ in the amount of environmental stress they experience, and this may influence the ability of the trees at the two sites to provide photosynthate to their ectomycorrhizal mutualists following herbivory. Pinyons growing in sandy loam soils have higher growth rates (Gehring and Whitham 1994b), lower levels of water stress (Mopper et al. 1991), and higher levels of cone production (Christensen and Whitham 1991) than pinyons growing in cinder soils, most likely due to higher levels of nutrients and water in this soil (Gehring and Whitham 1994b). Better conditions in sandy loam environments could enable pinyons growing there to provide sufficient photosynthate to sustain normal levels of ectomycorrhizae following short-term herbivory.

Third, differences in overall levels of ectomycorrhizal colonization between pinyons growing in cinder and sandy loam soils could lead to differences in the carbon costs of maintaining ectomycorrhizae at the two sites. Pinyons growing in sandy loam have 30–50% lower levels of ectomycorrhizal colonization than pinyons growing in cinders (Gehring and Whitham 1994b), and may therefore have lower ectomycorrhizal

carbon costs. In sandy loam, where fewer ectomycorrhizae are supported, pinyons may be able to lose tissue to herbivores and still provide sufficient energy to maintain their lower levels of ectomycorrhizae.

Our finding that environmental stress may influence ectomycorrhizal responses to herbivory could have important consequences for plants experiencing global warming. Our results suggest that if plants experience greater moisture stress due to increased environmental temperatures, they would be more likely to experience herbivory (Mopper et al. 1991, Gehring and Whitham 1994b) and to suffer mycorrhizal reductions as a consequence of herbivory. These effects would be particularly important in plants growing in arid environments where even small increases in temperature could have a detrimental effect on a plant's ability to maintain water balance.

*Effects of duration of herbivore
removal on ectomycorrhizae and conelet
production at a cinder site*

Removal of moths from chronically attacked susceptible trees for 1 yr had no effect on levels of ectomycorrhizal colonization, while removal for 10 yr resulted in significant ectomycorrhizal recovery. Ectomycorrhizae showed similar increases to those observed in our 10-yr-removal trees following 7 yr of herbivore removal (Gehring and Whitham 1991), suggesting that ectomycorrhizal recovery takes from 2 to 7 yr. In contrast, conelet production increased significantly following only 1 yr of moth removal.

The slower response of ectomycorrhizae relative to conelets could be due to reductions in the quantity of mycorrhizal inoculum beneath susceptible trees. Because *Dioryctria* attacks the same trees year after year, the mycorrhizae of these trees are also chronically reduced, and levels of mycorrhizal inoculum (e.g., active root tips, spores) may be reduced as well. In addition, the small cone crops characteristic of susceptible trees are associated with reduced cone-harvest rates by rodents (Christensen and Whitham 1993). Rodents are important dispersers of mycorrhizal inoculum in other coniferous forests (Maser et al. 1978), and their reduced foraging on moth-susceptible trees could reduce the quantity of ectomycorrhizal inoculum beneath these trees. This hypothesis predicts that rodent foraging would increase on the insect-removal trees as their cone crops increased, eventually leading to increased quantities of ectomycorrhizal inoculum.

A second hypothesis for the lack of a rapid recovery in ectomycorrhizal colonization following herbivore removal is that conflicting energy demands may prevent trees from providing sufficient photosynthate to ectomycorrhizal fungi. Lingering effects of herbivory were observed in vesicular-arbuscular mycorrhizal (VAM)-colonized wheat, presumably due to grazing-induced carbon limitation that reduced VAM colonization and grain yield (Trent et al. 1988). Mycorrhizae

are hypothesized to be costly to plants, with an estimated 10–60% of a plant's photosynthate going to these mutualists (Fogel and Hunt 1979, Harris and Paul 1987). Cone production in pinyons is also an energetically expensive process, but this cost may be partially offset by photosynthesis of the developing cone (Floyd 1987). For example, pinyon cones prevented from photosynthesizing were 44% smaller than photosynthetic cones (Floyd 1987).

In conclusion, the patterns observed in this study may help explain why other studies examining mycorrhizae–plant–herbivore relationships have been so varied. For example, our results suggest that, in response to herbivory, plants growing in stressful environments are more likely to experience reductions in mycorrhizal colonization than plants growing in areas richer in water and nutrients. We have also shown that ectomycorrhizal levels did not rebound immediately following herbivore removal, while conelet production rapidly returned to near normal levels. This suggests that conelet production and ectomycorrhizal colonization are not tightly linked in this system.

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